

Product datasheet for **TA347152**

H3FA (HIST1H3A) Mouse Monoclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	ELISA, IF, WB
Recommended Dilution:	ChIP/ChIP-seq (1 µg/ChIP) ; ELISA (1:5,000); Western blotting (1:1,000); Immunofluorescence (1:500)
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	The immunogen for anti-H3K4me3 antibody: histone H3, trimethylated at lysine 4 (H3K4me3), using a KLH-conjugated synthetic peptide
Concentration:	lot specific
Purification:	Protein A purified monoclonal antibody in PBS containing 0.05% azide.
Conjugation:	Unconjugated
Storage:	Store at -20°C as received.
Stability:	Stable for 12 months from date of receipt.
Gene Name:	histone cluster 1, H3a
Database Link:	NP_003520 Entrez Gene 8350 Human P68431

Background: Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble and are wrapped by 146 base pairs of DNA to form one octameric nucleosome. Histone tails undergo numerous post-translational modifications, which either directly or indirectly alter chromatin structure to facilitate transcriptional activation or repression or other nuclear processes. In addition to the genetic code, combinations of the different histone modifications reveal the so-called "histone code". Histone methylation and demethylation is dynamically regulated by respectively histone methyl transferases and histone demethylases.

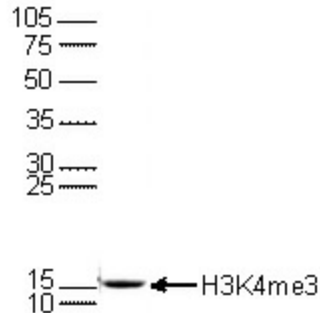


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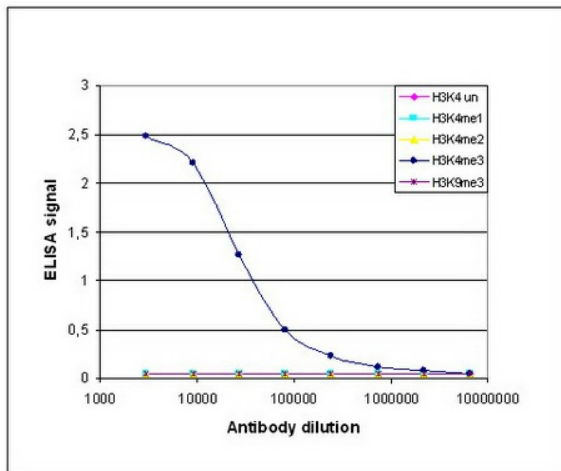
Synonyms: A; H3; H3FA

Protein Pathways: Systemic lupus erythematosus

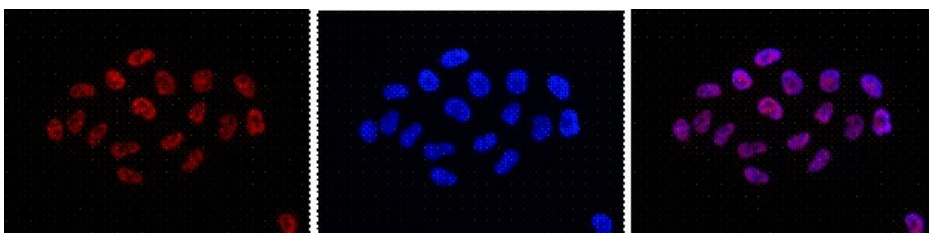
Product images:



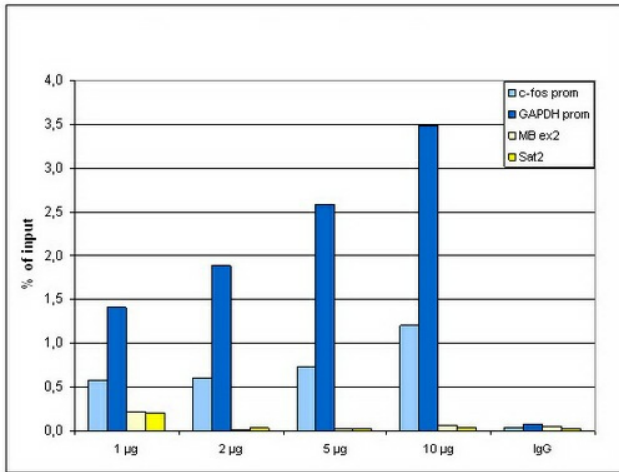
WB using the antibody against H3K4me3 diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



Cross reactivity of the antibody against H3K4me3
 To test the specificity an ELISA was performed using a serial dilution of the antibody against H3K4me3. The wells were coated with peptides containing the unmodified H3K4 as well as the mono-, di- and trimethylated H3K4 and the trimethylated H3K9. Image shows a high specificity of the antibody for the modification of interest.

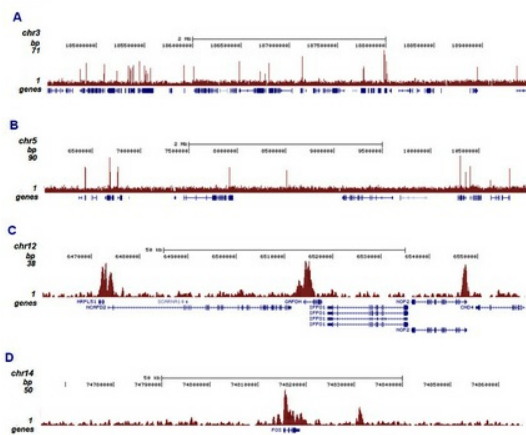


HeLa cells were stained with the antibody against H3K4me3 and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K4me3 antibody (left) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.



ChIP assays using HeLa cells: ChIP-seq[™] kit, using sheared chromatin from 1 million cells. A titration of 1, 2, 5 and 10 µg ab was used (2µg/IP IgG as negative control). qPCR primers were specific to the promoter of constitutively expressed GAPDH and c-fos genes as positive controls, and for exon 2 of the inactive myoglobin (MB) gene and the Sat2 satellite repeat as negative controls. Image shows the recovery, expressed as a % of input (the relative amount of IP'd DNA compared to input DNA after qPCR): results in accordance with the observation that trimethylation of K4 at histone H3 is associated with the promoters of active genes.

Figure 2



ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 2 µg H3K4me3 ab. The IP'd DNA was analysed on an Illumina Genome Analyzer. The 36 bp tags were aligned to the human genome using the ELAND algorithm. Image shows the peak distribution along two 5 Mb regions of chromosome 3 and 5 and in two 100 kb regions surrounding the GAPDH and c-fos positive control genes (C and D). These results clearly show an enrichment of the H3K4 trimethylation at the promoters of active genes.